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Adipocyte fatty acid binding protein (FABP4) is a 132-aa intracellular lipid binding protein involved in the transport of fatty acids between cell membranes and organelles. FABP4 participates in several pathways including lipolysis and lipogenesis, and is involved in lipid and energy metabolism related diseases such as diabetes. Additionally, in animal models, inhibitors of FABP4 have been found to halt the progression of diabetes that is usually concurrent with obesity. Although the structure of FABP4 has been determined using x-ray crystallography and binding to several of its hydrophobic ligands well characterized, the transitions in the structural dynamics upon ligand binding have yet to be investigated. Here, we report the results of the NMR resonance assignment of the apo form of human FABP4, and compare these results to chemical shift prediction analysis performed using SHIFTX and SPARTA. We also report spin relaxation measurements used to probe the fast (ps-ns) backbone dynamics. Finally, through NMR titrations, we are investigating the structural dynamics transitions which occur upon binding of FABP4 to its hydrophobic ligands, including several novel lipid ligands. The project is a working collaboration between St. Catherine University, the University of Minnesota, and the Minnesota NMR Center, and provides a model of conducting collaborative undergraduate research in partnership between a PUI, a major research institution, and an instrument center.

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Solid State NMR Structural Studies of Antimicrobial Peptides LPcin Analogs with Enhanced Activities

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The availability of antibiotics has allowed for the successful treatment of many bacterial infections as well as the ability to perform invasive medical procedures including surgery and chemotherapy. However, their wide use has led to pathogens' increased drug resistance and the need to find novel classes of antimicrobial peptides as alternatives to antibiotics. Lactophoricin (LPcin), a cationic amphipathic peptide consists of 23-mer peptide, corresponds to the carboxy terminal 113-135 region of component-3 of proteose-peptone. LPcin is a good candidate as a peptide antibiotic because it has an antibacterial activity but no hemolytic activity. Three different analogs of LPcin, LPcin-yk2 which has mutant amino acids, LPcin-yk1 and LPcin-yk3 that has shorter mutant amino acids are recently developed by using peptide engineering in our laboratory. These three LPcin analogs show better antibioitic activities than wild-type LPcin and no toxicity at all.

In order to understand the structural correlation between LPcin analogs structure and antimicrobial activity under the membrane environments, we tried to express and purify as large as amounts of LPcin and three different LPcin analogs. We finally optimized and succeed to overexpress in the form of fusion protein in Escherichia coli and purified with biophysical techniques like Ni-affinity chromatography, dialysis, centrifuge, chemical cleavage, and reversed-phase semiprep HPLC. In here, we will present the optimizing processes with high-yield expression and purification of three LPcin analogs and solid- state NMR structural studies to figure out antibacterial killing mechanisms.

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NMR-Restrained Structure Calculations of Membrane Proteins in Implicit Lipid Bilayer Membranes

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The biological functions and molecular structures of proteins are highly dependent on the physical and chemical properties of the surrounding environment. Just as water is essential for supporting the native states of soluble proteins, the lipid bilayer is critical for preserving the functional and structural integrity of membrane proteins.

The principal advantage of NMR spectroscopy as a method for structure determination is its ability to examine proteins in samples that are very close to their functional environments. NMR is well suited for studying soluble proteins in water and membrane proteins in lipid or detergent environments.

However, NMR structure calculations in explicit solvent or explicit lipids are computationally expensive and many NMR structures are calculated with a simplified repulsive term to prevent atom clashing. This accelerates the calculation but sacrifices analysis of the non-bonded interactions that play important roles in structure and function. To facilitate NMR structure calculations in a physically realistic environment we are developing a computationally efficient implicit solvent and implicit membrane potential for the Xplor-NIH, NMR structure refinement package.

Here we show that the potential provides significant improvements both in the quality and precision of the calculated structures, provides correct embedding of membrane proteins in lipids, and provides physically meaningful views of residue-residue interactions and residue-membrane interactions.

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Global Fold of Human Cannabinoid Type 2 Receptor Probed by Solid-State NMR and Molecular Dynamics Simulations

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Uniformly 13C- and 15N-labeled receptor in milligram quantities was produced by bacterial fermentation, purified and functionally reconstituted into unilamellar liposomes in the agonist-bound state. 13C- and 15N-NMR spectra of the labeled CB2 were recorded by solid-state, magic-angle-spinning (MAS) NMR. The structure of CB2 was obtained by homology modeling to rhodopsin, followed by energy minimization and MD simulations. Microsecond-timescale molecular dynamics simulations of CB2 in a lipid bilayer gave insights into molecular details of receptor activation upon agonist binding. The atomic coordinates of CB2 were used for prediction of chemical shifts of resonances before and after CB2 activation using the programs SHIFTX and SPARTA. Experimental and model-derived Cα, Cβ, C=O, and N-H chemical shifts of amino acids were compared. The chemical shifts of the Cα region of the protein are in reasonable agreement between measurement and prediction from the molecular model confirming that secondary structure prediction from the model agrees reasonably well with experimental reality. Activation of CB2 upon ligand binding is predicted to result in significant changes of chemical shifts of a small number of resonances located primarily in N-terminal domain, extracellular loop 2, the second half of intracellular loop 3, and the first half of C-terminal domain. Amino acid residues in those regions are desired targets for specific amino-acid labeling to study mechanisms of receptor activation.

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BCL-2 Family Proteins Effect on Mitochondrial-Mimicking Membrane Structure by Solid State NMR

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Mitochondria are not only the cells' powerhouse, but also involved in their suicide via apoptosis. Key regulators of this pathway are members of the Bcl-2 protein family which interact with the outer mitochondrial membrane to modulate permeability and enable the release of apoptotic stimuli like cytochrome c. For a long time the mitochondrial membrane forming lipids have been seen as merely structural building units with proteins doing the actual work. This view changed in recent years, since lipids were shown to be also directly involved in apoptotic events e.g. under intracellular oxidative stress. Oxidized phospholipids (OxPls) generated under these stress conditions might trigger mitochondria-mediated apoptosis. Their presence in mitochondrial membranes can severely alter the properties of these membranes with yet unknown consequences regarding the formation of pores through membrane-mediated interplay with apoptotic Bax protein. We therefore devised a model system that embodies oxidative stress conditions by incorporating OxPls into mitochondria mimicking model membranes composed of phosphatidylcholine (PC), phosphatidylethanolamine (PE) and cardiolipin (CL) to study the impact of OxPls on apoptotic Bax-membrane interactions. To obtain molecular insight into hydrophobic fatty acid regions of membranes and their hydrophilic interface which is responsible for first protein-membrane contacts, we used differential scanning calorimetry (DSC) and solid state NMR spectroscopy. Upon incorporating OxPls with carboxyl (PoxnoPC) or aldehyde (PazePC) groups at their truncated sn-2-chains into our mitochondria model membranes, calorimetric and NMR measurements showed dramatic changes. 31P NMR experiments revealed major perturbation effects in these membranes; an effect which presumably elevates the membrane binding of apoptotic Bax to the charged membranes and its partial penetration, being a prerequisite for its final formation of pores which enable cytochrome c release